Root Rot of Lettuce Incited by *Pythium polymastum*

D. L. COPLIN, A. F. SCHMITTHENNER, and W. L. BAUERLE, Assistant Professor and Professor, Department of Plant Pathology, and Associate Professor, Department of Horticulture, respectively, Ohio Agricultural Research and Development Center, Wooster 44691

ABSTRACT

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A *Pythium* sp. with large spiny oogonia has been consistently associated with root rot and decline of lettuce cultivar Grand Rapids H5-4 in northern Ohio. The disease is characterized by severe stunting and rosetting followed by wilting and death of the plant; lateral roots and the cortical tissue of the tap root are severely rotted. The lettuce root isolates are morphologically similar to *P. polymastum* and can be identified by their large oogonia (average diameter 45 μm) with conical spines ranging from 3 to 7 μm in length. The fungus is slow growing and difficult to isolate. In pathogenicity tests on lettuce, the isolates with spiny oogonia were consistently more virulent than other *Pythium* spp. isolated from lettuce roots. In greenhouse experiments, escarole, endive, and the lettuce cultivars Salad King and Romaine were resistant to root rot and Grand Rapids H5-4, Slobolt, and Boston were susceptible.

Additional key words: *Lactuca sativa*, soilborne pathogens

Lettuce (*Lactuca sativa* L.) is susceptible to a variety of root diseases attributed to *Pythium* spp. (7). The etiology of these diseases has not always been completely understood. During the last 3 yr, a *Pythium* sp. with large, spiny oogonia has been consistently associated with root rot and decline of lettuce cultivar Grand Rapids H5-4 in northern Ohio. This disease has been particularly severe in fields where several crops of lettuce were grown successively during the season. Almost all plants in the third consecutive crop showed signs of root damage, and yield losses ranged from 25 to 50%.

Symptoms of lettuce root rot include chlorosis, severe stunting, and rosetting of the plants, and the lower leaves frequently wilt and die. Aboveground symptoms are attributable to poor root development and cortex decay. The main tap root shows varying degrees of decay from brownish discoloration of the epidermis to rotting of the cortical tissue. Lateral roots rot or fail to develop, but vascular tissues and pith are seldom visibly affected. In this paper, we report that lettuce root rot in Ohio is caused by a fungus tentatively identified as *P. polymastum*.

MATERIALS AND METHODS

Media and growth of fungi. Isolates of *Pythium* spp. were maintained on V8 juice-yeast extract-sucrose (VSY) agar (9) at 23 C. Initial isolations were made on VSY medium supplemented with pentachloronitrobenzene, benomyl, neomycin, and chloramphenicol (VSY-PBNC) (9). Hemp seed or lima bean semisolid media were used to produce inoculum for pathogenicity studies. These media were prepared by autoclaving 50 g of hemp seeds or lima beans in 1 L of water for 30 min at 121 C. The supernatant was decanted, Difco Bacto agar was added at 2 g/L, and 15-ml aliquots were dispensed into sterile petri dishes.

Microscopic examination of root tissue. Hand-cut sections of infected roots were cleared and stained by boiling for 10 min in chloral hydrate-acid fuchsin stain (5). Oospores were visible in root tissues by light microscopy at ×160.

Pathogenicity tests. Commercial 2-wk-old lettuce seedlings were transplanted into tomato baskets (four plants per 55 × 38 × 8 cm basket) or flats (eight plants per 40 × 30 × 12 cm flat) in a 4:4:1 mixture (v/v) of Wooster silt loam, peat, and muck. Plants were grown in a greenhouse with vertical pad cooling at 23 C and supplemented with warm white fluorescent lighting (15,000 lux with a 14-day length).

Seedlings were inoculated 2–3 days after transplanting. One-wk-old cultures of *Pythium* sp. growing on hemp seed or lima bean semisolid media were ground in a Waring Blendor at high speed for 10–15 sec and then diluted with an equal volume of tap water. This produced a suspension containing about 2,000–3,000 viable units per milliliter. The suspension was then injected into the soil at two positions 1 cm from the base of the tap root, using a syringe with a 12- or 15-gauge needle. Each plant received either 5 or 10 ml. After 30 days the plants were uprooted and washed. The amount of root damage on each plant was rated on a scale of 1 (healthy) to 5 (severely stunted and rotted).

Temperature studies were done in controlled-environment chambers at day temperatures of 21 and 30 C and respective night temperatures of 13 and 18 C. A mixture of cool white fluorescent and incandescent lighting (23,000 lux) with a 16-hr photoperiod was used. Lettuce seedlings were grown in baskets and inoculated as described except they were held in the chamber for 1 wk before inoculation. Treatments consisted of four replications of five plants each.

RESULTS

Isolation of *Pythium* spp. Initial microscopic observations of infected root tissue revealed numerous large oospores with spiny protuberances (Fig. 1). A *Pythium* sp. identified as *P. polymastum* with oospores identical to those observed in infected tissue was isolated by plating large pieces of washed, surface-sterilized roots on VSY agar. Most samples yielded *P. ultimum* Trow., *P. irregulare* Buis., *P. vexans* de Bary or one of several unidentified species with spherical sporangia that did not produce oospores. All these species grew much faster than *P. polymastum*, especially on VSY-PBNC agar, and when present in roots they interfered with its isolation. Over a 2-yr, period, we were able to isolate *P. polymastum* from only seven of 31 samples in which spiny oogonia were observed.

Characteristics of *P. polymastum* isolates from lettuce. On 1-wk-old grass leaf cultures, *P. polymastum* isolates produced oogonia ranging in diameter, excluding spines, from 36 to 53 μm (average 45 μm). Spines were conical and ranged in length from 3 to 7 μm and in width at the base from 2 to 4 μm. Sporangia were spherical, 14–46 μm in diameter (average 36 μm), and either terminal or intercalary. No sporangial germination was observed in grass leaf cultures after chilling at 11 C for 0.5 hr. When lettuce seed were added to these cultures, however, the fungus invaded germinating seedlings and produced additional sporangia. After 48 hr, these cultures were leached with continuously changing deionized water for 1 hr at a flow rate of 4 ml/min, and a small number of sporangia germinated. The evacuation tube was about the same length as the diameter of the oogonium, and zoospores were about 13 μm in

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Table 1. Pathogenicity of *Pythium* isolates from lettuce on cultivar Grand Rapids H5-4

Isolate	Average disease index[v]		
	Experiment 1[w]	Experiment 2[x]	Experiment 3[y]
P. polymastum L4	3.9 b	3.2 c	3.2 c
P. polymastum L5	4.0 b		
P. vexans	1.9 a	1.4 a	
P. irregulare	1.8 a	2.3 b	
P. ultimum		1.2 a	1.7 a
Pythium sp. L2	1.9 a	1.4 a	
Pythium sp. L13	1.7 a	1.6 a	
Pythium sp. L14	2.4 a	1.2 a	
P. mastophorum[z]		1.2 a	1.3 a
P. polymastum[z]		2.5 b	2.5 b
Uninoculated control	1.7 a	1.2 a	1.4 a

[v] Disease index: 1 = no evidence of root rot; 2 = tap root discolored, lateral roots reduced up to 20%; 3 = tap root and lateral roots rotted, lateral root mass reduced up to 50%, plants slightly stunted; 4 = tap roots and lateral roots severely rotted, lateral root mass reduced up to 80%, moderate stunting; 5 = tap root severely rotted, few or no lateral roots, plants severely stunted. Means in the same column followed by different letters are significantly different at $P = 0.05$ using Duncan's multiple range test.

[w] Average of five plants; one-way analysis of variance.

[x] Randomized block design with four replicates of five plants each; two-way analysis of variance.

[y] Completely randomized design with four replicates of five plants each; one-way analysis of variance.

[z] Obtained from Commonwealth Mycological Institute.

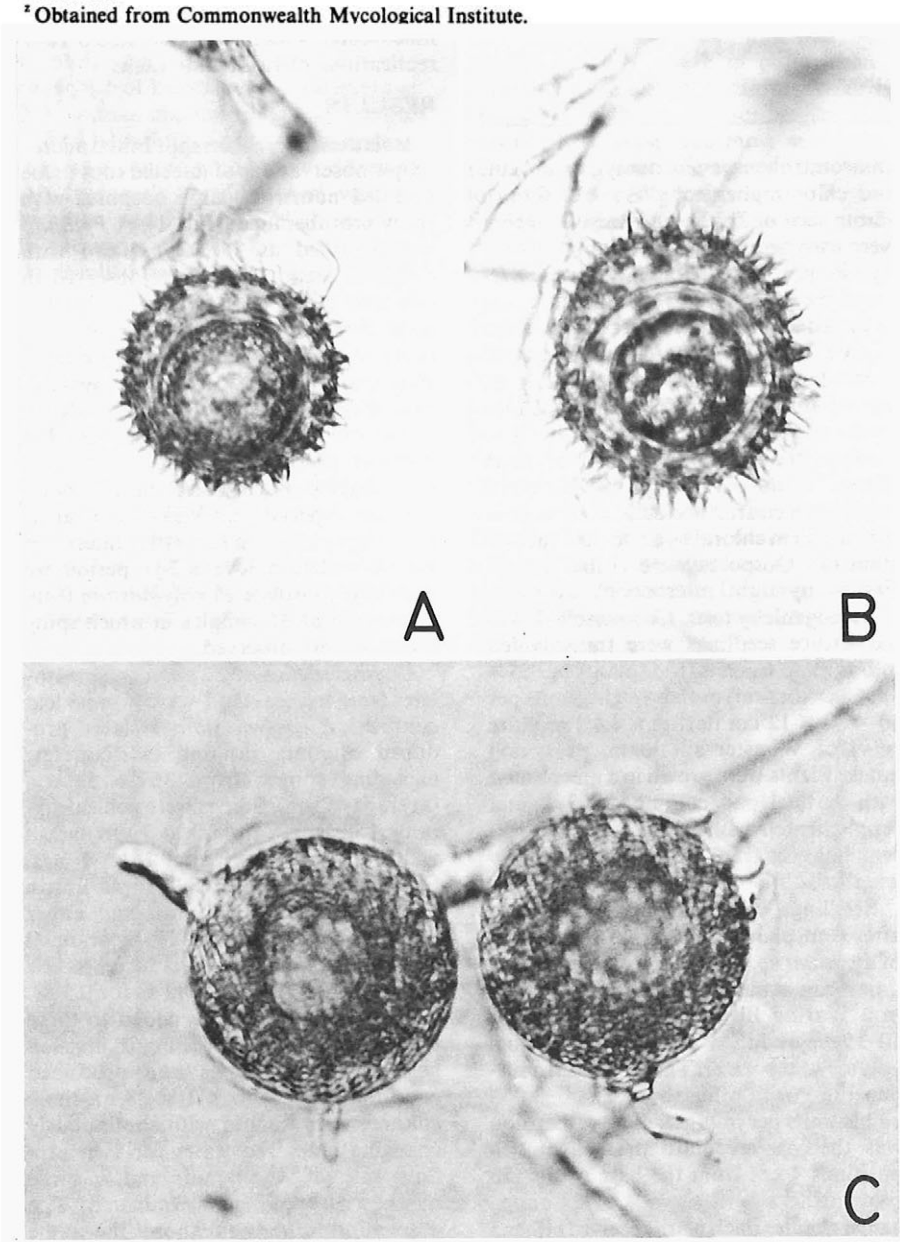

Fig. 1A–C. Oogonia and sporangia produced by *Pythium polymastum* isolate L4: **(A)** oogonium with attached antheridium, **(B)** oogonium, and **(C)** sporangia.

diameter. Oogonia on Difco corn meal agar and lima bean semisolid medium were similar to those on grass leaf cultures, but sporangia were rarely produced.

Pathogenicity of isolates from lettuce. Pathogenicity of two *P. polymastum* isolates, L4 and L5, was compared with that of *P. vexans*, *P. irregulare*, *P. ultimum*, and three unidentified *Pythium* spp., all isolated from diseased lettuce. In three experiments (Table 1), the two *P. polymastum* isolates were highly virulent on lettuce cultivar Grand Rapids H5-4. Symptoms produced by these isolates (Fig. 2) were identical to those observed in the field. Stunting, wilting, loss of lateral roots, and decay of the cortex of the main tap root occurred. In contrast, plants inoculated with other *Pythium* spp. isolated from the same field samples were not significantly different from the control, except in one instance where a *P. irregulare* isolate caused slight damage (Table 1). A known isolate of *P. mastophorum* Drechs., obtained from the Commonwealth Mycological Institute (CMI), was slightly virulent and a known isolate of *P. polymastum* Drechs., also obtained from CMI, was avirulent (Table 1). Roots from plants inoculated with isolates L4 and L5 were cleared, stained, and examined microscopically. In all cases, large spiny oogonia were observed in the tissues and *P. polymastum* was reisolated.

Infected plants from selected treatments in Experiment 2 (Table 1) were pulled up and the flats replanted with healthy seedlings. After 4 wk, plants in soil previously infested with isolate L4 and the known isolate of *P. polymastum* showed significantly more root rot than those growing in the flats infested with *P. irregulare* or in uninfested soil. Disease indexes of plants in these soils were 2.0, 1.7, 1.1, and 1.0, respectively (LSD, $P = 0.05, = 0.4$).

The pathogenicity of *P. polymastum* L4 to seedlings of Grand Rapids H5-4 did not appear to be affected by temperature in tests at 70 and 85 C (daytime temperature); disease indexes were 3.0 ± 0.3 and 3.0 ± 0.7, respectively. Uninoculated controls did not show any symptoms.

Susceptibility of lettuce cultivars to *P. polymastum*. Susceptibility of five lettuce cultivars, endive (*Cichorium endivia*), and escarole (*C. endivia*) to *P. polymastum* L4 was tested in two experiments (Table 2). In Experiment 1, inocula (prepared as previously described) were used undiluted and after tenfold dilution. In both experiments, escarole, endive, Salad King, and Romaine were resistant and Grand Rapids H5-4, Slobolt, and Boston were susceptible. Roots of resistant plants were affected slightly, but the plants were not stunted or wilted.

DISCUSSION

Symptoms of lettuce root rot are

similar to those produced by overfertilization (6) and corky root rot (1). The latter disease is caused by incorporation of crop residues into soil just before planting, occurs on wet muck soils, and becomes worse with successive croppings. In Ohio, however, root rot occurs primarily on sandy loam soils where tests indicate normal amounts of nitrogen. Furthermore, we have been unsuccessful in reproducing the symptoms of this disease in greenhouse experiments by incorporating lettuce debris from healthy greenhouse-grown plants into nonsteamed soil collected near a problem field. The results of the present study clearly show that a *Pythium* sp. with large spiny oogonia, which we have identified as *P. polymastum*, is often responsible for root rot of lettuce.

Since the Ohio isolates of *P. polymastum* from lettuce in some way resemble each of the three species of *Pythium* with oogonial spines (*P. polymastum*, *P. megalacanthum* de Bary, and *P. mastophorum*) described by Drechsler in 1939 (4), our identification of this pathogen must be qualified. *P. polymastum* was originally isolated from lettuce in Connecticut in 1921. Drechsler, however, was unable to isolate this fungus again, although he examined rotted lettuce plants over a period of 18 yr. Recently, Vanterpool (11) reported isolation of *Pythium* spp. with spiny oospores from crucifers that he identified as *P. polymastum*. These isolates, however, were only weakly pathogenic on lettuce. Tasugi and Siino (10) reported isolates of *P. megalacanthum* from *Callistephus chinensis* (China-aster) that caused damping-off of lettuce. We are not aware of any reports of the pathogenicity of *P. mastophorum* to lettuce.

The Ohio isolates differ from *P. mastophorum* in having larger oogonia with conical spines and a short, straight, unbranched, sporangial evacuation tube. They differ slightly from the original description of *P. polymastum* in having spherical sporangia without the irregularities in shape, such as connected lobes or dome-shaped diverticula, emphasized by both Drechsler (4) and Middleton (8). Furthermore, the Ohio isolates have conical oogonial spines, whereas those described for *P. polymastum* are largely mammiform. The Ohio isolates, however, are similar to the description of *P. polymastum* with respect to size of oogonia and zoospores and the type of sporangial evacuation tube. They are similar to the flax isolates of *P. megalacanthum* studied by Drechsler (4) with respect to oogonial spine characteristics but have smaller oogonia. Proliferation of sporangia, considered definitive for *P. megalacanthum* by Middleton (8), was not observed with *P. polymastum* from Ohio. In identifying his isolates from oilseed rape as *P. polymastum*, Vanterpool (11) decided that oogonial

Table 2. Pathogenicity of *Pythium polymastum* isolate L4 on escarole, endive, and cultivars of lettuce

	Average disease index[w]		
	Experiment 1[x]		Experiment 2[y]
Cultivar	High inoculum[z]	Low inoculum	High inoculum
Slobolt	4.2 bc	4.0 d	3.4 c
Grand Rapids H5-4	4.8 c	2.4 c	
Boston	3.3 b	2.0 bc	2.7 bc
Romaine	2.0 a	1.6 ab	2.2 b
Salad King			1.3 a
Endive	1.4 a	1.0 a	
Escarole	1.0 a	1.2 a	1.2 a

[w] Disease index: 1 = no evidence of root rot; 2 = tap root discolored, lateral roots reduced up to 20%; 3 = tap root and lateral roots rotted, lateral root mass reduced up to 50%, plants slightly stunted; 4 = tap roots and lateral roots severely rotted, lateral root mass reduced up to 80%, moderate stunting; 5 = tap root severely rotted, few or no lateral roots, plants severely stunted. Means in the same column followed by different letters are significantly different at $P = 0.05$ using Duncan's multiple range test.

[x] Average of five plants; one-way analysis of variance.

[y] Completely randomized design with four replicates of five plants each.

[z] High and low inoculum concentrations were 2,500 and 250 propagules per milliliter, respectively.

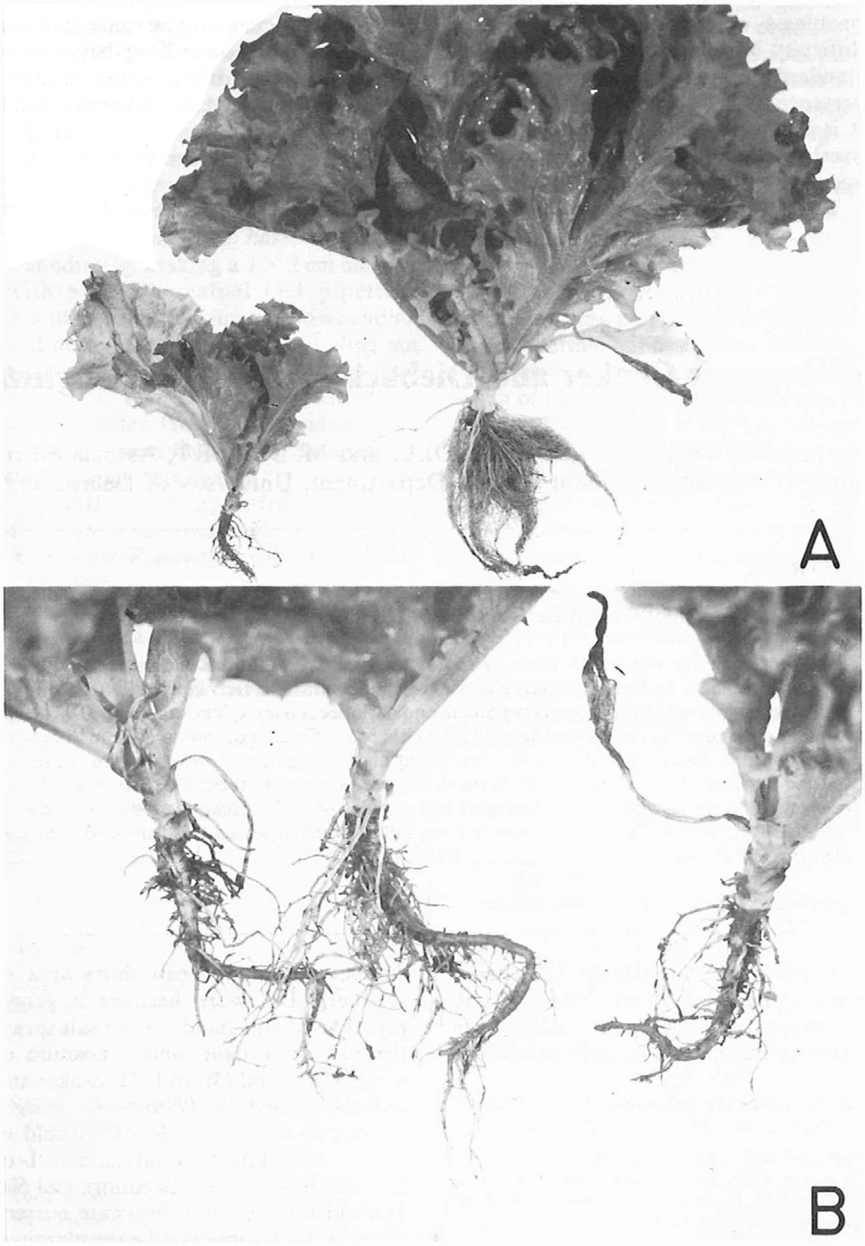

Fig. 2A,B. Lettuce plants (cultivar Grand Rapids H5-4) 30 days after inoculation with *Pythium* isolate L4: **(A)** Plant on left shows severe stunting (disease index = 5); plant on right is uninoculated control (disease index = 1). **(B)** Close-up of root damage.

spine characteristics were not sufficient for species separation; he made no mention of sporangial irregularities. The Ohio isolates are similar to Vanterpool's description of *P. polymastum* (11) but have more oogonial spines than shown in his figures. In designating the lettuce root rot pathogen as *P. polymastum*, we have chosen to follow Vanterpool's lead and minimize the importance of oogonial spine shape and sporangial irregularities.

Symptoms produced by *P. polymastum* are very similar to those of *Pythium* stunt of lettuce, a disease reported 60 years ago in New York and Michigan (2,3,7). The exact cause of stunt has never been determined, although *P. vexans*, *P. ultimum*, and *P. debaryanum* have been isolated from diseased tissue during the early stages of disease development (7). We are not aware of any *Pythium* sp. with spiny oogonia that has been associated with stunt or similar problems of lettuce. Because of the difficulty in isolating *P. polymastum* by standard techniques, especially in the presence of faster growing *Pythium* spp., it is likely that this pathogen has been overlooked and may in fact be the causal agent of lettuce stunt.

The lettuce root *Pythium* is soilborne and can overwinter in Ohio, as evidenced by the occurrence of root rot for three consecutive years in a field planted with pathogen-free, greenhouse-grown transplants. The appearance of the disease in late summer is not characteristic of diseases caused by *Pythium* spp. that are usually favored by cool, wet weather. Using controlled-environment chambers, we were unable to detect a significant difference in pathogenicity of *P. polymastum* at 21 C and 30 C day temperatures. Therefore, the increased severity of the disease in late summer may be due to an increase in inoculum density from continuous lettuce cropping. This is supported by the absence of the disease in fields where lettuce is rotated with other vegetable crops during the same season.

Control of lettuce root rot may be possible by rotating lettuce with other crops, removing infested lettuce residues from fields when practical, and using tolerant varieties. In this study, cultivars Romaine and Salad King have shown promising levels of resistance in greenhouse tests but have not yet been tested in the field.

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